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**VENOUS MALFORMATIONS:  
DIFFERENTIAL DIAGNOSTICS, BLOOD  
COAGULATION, AND TREATMENT SAFETY**

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Academic Dissertation

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To all children with venous malformations





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## List of original publications

- (I) Aronniemi J, Lohi J, Salminen P, Lappalainen K, Vuola P, Pitkäranta A, Pekkola J, Angiomatosis of soft tissue as an important differential diagnosis for intramuscular venous malformations. *Phlebology*. 2016 Sept 29. Doi 10.1177/0268355516671463. Reprinted by permission of SAGE Publications.
- (II) Aronniemi J, Mattila K, Mäkipernaa A, Salminen P, Pitkäranta A, Pekkola J, Lassila R, Venous malformations and blood coagulation in children. Submitted to *Journal of Thrombosis and Haemostasis*.
- (III) Aronniemi J, Castren E, Lappalainen K, Vuola P, Salminen P, Pitkäranta A, Pekkola J, Sclerotherapy complications of peripheral venous malformations. *Phlebology*. 2015 Oct 22. Doi 10.1177/0268355515613740. Reprinted by permission of SAGE Publications.
- (IV) Castren E, Aronniemi J, Klockars T, Pekkola J, Lappalainen K, Vuola P, Salminen P, Pitkäranta A, Complications of sclerotherapy for 75 head and neck venous malformations. *Eur Arch Otorhinolaryngol*. April 2016, Volume 273, Issue 4, pp 1027–1036. Reprinted by permission of Springer.

The studies are referred to in the text by their Roman numerals.

## **Abbreviations**

AST: Angiomatosis of soft tissue

AT: Antithrombin

AVF: Arteriovenous fistula

AVM: Arteriovenous malformation

BRBNS: Blue rubber bleb nevus syndrome

cedMRI: Contrast-enhanced dynamic magnetic resonance imaging

CLVM: Capillary-lymphatic-venous malformation

CVM: Capillary-venous malformation

DIC: Disseminated intravascular coagulation

FAVA: Fibro-adipose vascular anomaly

HUH: Helsinki University Hospital

ICD: International Classification of Diagnoses

ISSVA: International Society for the Study of Vascular Anomalies

LIC: Local intravascular coagulopathy

LMWH: Low-molecular-weight heparin

LVM: Lymphatic-venous malformation

MRI: Magnetic resonance imaging

PHOST: PTEN hamartoma of soft tissue

PTEN: Phosphatase and tensin homolog

STS: Sodium tetradecyl sulphate

US: Ultrasonography

VM: Venous malformation

## Abstract

**Background:** Venous malformations (VMs) are congenital vascular anomalies resulting from local defects in the vascular morphogenesis during fetal development. They cause highly variable symptoms and are associated with blood coagulation disorders. The treatment of VMs aims to alleviate symptoms, disfigurement, and blood coagulopathy. The current treatment strategies, including conservative treatment, sclerotherapy, surgery and laser ablation, all have different benefits and risk profiles. Sclerotherapy has recently become the primary treatment for most VMs. Sclerotherapy is, however, not without complications and some VMs seem refractory to it.

**Aims:** This thesis aimed to investigate histology and imaging findings of VMs operated on for unsatisfactory sclerotherapy response (I), blood coagulation and fibrinolysis activity in pediatric VM patients (II), and safety aspects of sclerotherapy for trunk and extremity (III) and head and neck (IV) VMs.

**Patients and methods:** In Studies I, III, and IV we included all consecutive VM patients who underwent sclerotherapy at Helsinki University Hospital between 2007 and 2013. We analyzed histology and imaging findings of extremity VMs operated on for poor response to sclerotherapy (n=102) (I) and sclerotherapy complications of all sclerotherapy-treated patients (n=202) (III, IV). In Study II we included pediatric VM patients with detailed laboratory results for blood coagulation and fibrinolysis activity at a steady clinical phase (n=62). We analyzed patients' coagulation status and correlations of abnormal coagulation biomarkers to clinical and imaging findings.

**Results:** Of the sclerotherapy-treated extremity VMs, 19 (19%) were operated on for insufficient sclerotherapy response. The number of intramuscular lower extremity lesions among operated VMs was 15. The histology of 13 of the 15 intramuscular VMs was not consistent with common VM, corresponding instead to angiomatosis of soft tissue (AST) (I). The imaging findings for common VMs and ASTs were overlapping (I). Pediatric VM patients had significant abnormalities in leukocyte levels and antithrombin, FVII, FVIII, and FXIII activities (II), in addition to previously reported commonly elevated D-dimer levels. Disseminated intravascular coagulation did not

occur and platelets were generally normal (II). The sclerotherapy complication rate per procedure was 13% for trunk and extremity VMs (III) and 10% for head and neck VMs (IV). Superficial location and use of ethanol increased the risk for local conservatively manageable complications (III). Severe complications, for which blood coagulopathy was a significant predisposing factor, occurred in seven procedures (1.6%) (III, IV).

**Conclusions:** Histology has an important role in the differential diagnostics of intramuscular VMs, as different histological entities require different treatment approaches (I). VMs are associated with a number of specific abnormalities in coagulation biomarkers, implying a close interrelation between coagulation and angiogenesis (II). Sclerotherapy for both peripheral and head and neck VMs is generally safe, but entails a risk for severe complications (III, IV).

**Key words:** angiomatosis of soft tissue, coagulopathy, complication, differential diagnostics, histology, interventional radiology, safety, sclerotherapy, surgery, vascular anomaly, venous malformation

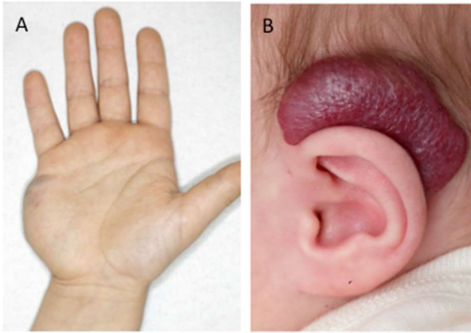
# 1. Introduction

The term ‘vascular anomaly’ refers to a wide spectrum of congenital malformations and tumors of vascular origin ranging from inconsequential birthmarks to life-threatening and severely disfiguring conditions. Throughout most of the recorded history, they have been believed to result from the emotions, thoughts, or desires of the expectant mother. In the mid-19<sup>th</sup> century, Scottish anatomist William Hunter questioned this theory of “maternal impression” and introduced the concept of vascular anomaly [1,2]. However, lack of systematic categorization of these diverse lesions held back medical progress for over a century. While mothers were no longer blamed, the limited diagnostic knowledge often led to inappropriate management and unnecessary anxiety.

In 1982, Mulliken and Glowacki studied the cellular characteristics of vascular anomalies and consequently divided them into vascular tumors and vascular malformations [3,4]. This division is still the basis for the current classification (ISSVA 2014 [5]) comprising a detailed taxonomy for dozens of different vascular entities.

This thesis focuses on venous malformations (VMs), which are the most common vascular malformations, giving rise to numerous consultations with interdisciplinary vascular anomaly teams [6,7]. VMs have highly variable clinical appearance and associations with the blood coagulation system [8]. Development of modern imaging modalities, radiological interventions, surgical techniques, and medications has essentially improved the management of VMs over the last two decades.

Lack of familiarity with the terminology and differential diagnostics nevertheless leads to confusion and even mismanagement of VM patients. The most common misnomer is ‘hemangioma’, which is incorrectly used in over 70% of publications concerning vascular anomalies [9]. ‘Hemangioma’ actually refers to endothelial tumors with cellular mitoses and proliferation [10] (Figure 1). VMs, instead, are structural anomalies with normal endothelial cell turnover [5,10]. The terms cavernous hemangioma (adult liver), venous hemangioma (soft tissue), and hemangioma of the bone (vertebra) should be abandoned as outdated and confusing, as these entities are histologically consistent with VMs [11-13].



**Figure 1.** Venous malformations (VMs) (A) are often confused with hemangiomas, of which infantile hemangioma (B) predominates and occurs only in children. When superficial, VM is bluish, soft, and compressible, whereas infantile hemangioma is relatively firm with a strawberry-like reddish appearance.

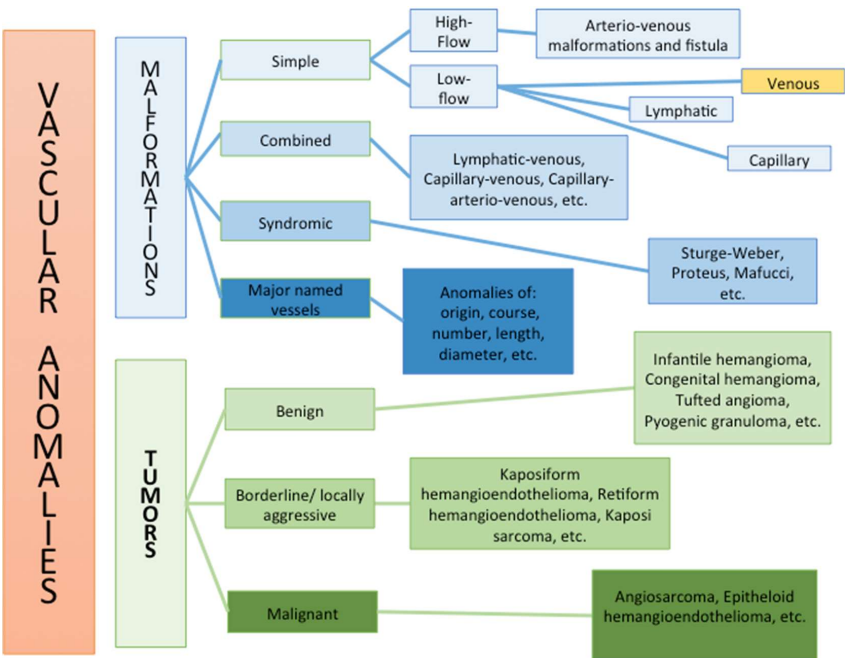
The classification of vascular anomalies is evolving as new entities are recognized and knowledge about their pathophysiology and genetics is refined. This continuous development challenges the clinical strategies in the treatment of VMs. The current treatment modalities for VMs, including medical therapy, surgery, laser, and sclerotherapy, are applied with good overall results [14]. Some VMs remain, however, challenging to manage. Studying their specific features may reveal obscure aspects in differential diagnostics and blood coagulation disorders and facilitate the selection of the optimal treatment for each VM patient.



## 2. Review of the literature

### 2.1 Classification of vascular anomalies

The classification of vascular anomalies aims to unify and clarify the complex terminology used by multidisciplinary professionals. Accurate terminology is crucial for achieving the right diagnosis, and thereby, proper management [9,15]. The first classification based on endothelial characteristics of the vascular anomalies was introduced by Mulliken and Glowacki in 1982 [4]. It has since been revised and expanded by the International Society for the Study of Vascular Anomalies (ISSVA) to include the latest knowledge about genetics and clinical associations [5,16]. The essential framework of the ISSVA classification is the division of vascular anomalies into congenital malformations and proliferative tumors [5,17] (Figure 2).



**Figure 2.** Outline of the current ISSVA classification of vascular anomalies [5].

Vascular malformations are structural defects resulting from local disturbances in the fetal vascular morphogenesis. Their further categorization is based on their predominant vessel type or combinations of different vessel types, as well as associations with other anomalies (Figure 2) [18]. Moreover, vascular malformations are often divided into low-flow and high-flow lesions for the clinical relevance of their pressure conditions (Figure 2) [19,20]. Vascular tumors, in turn, are proliferative lesions with mitotic activity [4]. They are divided into benign, borderline or locally aggressive, and malignant tumors according to their clinical behavior (Figure 2). Infantile hemangioma is the most common vascular tumor and also the most common pediatric tumor, affecting 4-10% of all children [1,21]. It has a unique clinical course of early proliferation and spontaneous involution (Figure 3). Congenital hemangioma, tufted angioma and kaposiform hemangioendothelioma are examples of rare vascular tumors with clinical course different from infantile hemangioma [22,23] (Figure 2).



**Figure 3.** Natural course of a nasal tip infantile hemangioma; early proliferative phase after birth is followed by spontaneous involution by school age. During the involution the vascular component gradually diminishes ending up as a fibro-fatty residual with some excessive skin.

## 2.2 Venous malformation

### 2.2.1 Definition

Venous malformations (VMs) are congenital structural defects of the postcapillary vessels with low-flow conditions [24,25]. They are typically local and singular lesions

affecting a restricted area of the body, but may also occur as segmental or multifocal (Figure 4). The anomalous veins may form local clusters or infiltrate diffusely any tissue or organ. They may be located superficially in subcutaneous fat or invade deep tissues such as muscle, bone or viscera [26].



**Figure 4.** Clinical appearance of VMs varies depending on the location, size, depth, and morphology of the lesion. While some VMs are totally asymptomatic, others with critical locations or bulky structures may cause severe functional impairment and disfigurement. Extremity VMs typically induce pain and swelling provoked by physical activity.

### 2.2.2 Genetics

The vast majority of VMs occur sporadically, with inherited disease forms comprising only 1-6% [27]. The sporadic VMs are mostly isolated or segmental malformations, whereas the familial forms typically consist of multifocal lesions [28,29]. The causative gene for the sporadic VMs in most cases is TEK, which encodes the endothelial cell tyrosine receptor kinase 2 (TIE2) [29-31]. It binds angiopoietins and participates essentially in angiogenesis regulation and vessel wall stability maintenance through the PI3K/AKT signaling pathway [30]. The second most common causative gene, PIK3CA, is found in 20% of the VMs and influences the same PI3K/AKT pathway as

TEK [32]. In addition to the common VM, the rare blue rubber bleb nevus syndrome (BRBNS) also occurs sporadically. BRBNS patients have TIE2 mutations as well, but they appear characteristically in double in the same allele [27,33,34].

Two different familial forms of VMs have been identified, the more common cutaneomucosal VM (VMCM) and the rare glomuvenous malformation (GVM) [27]. Both are inherited with an autosomal paradiominant pattern, necessitating a second hit mutation for lesion formation [27]. In VMCM the primary mutation is again in TEK/TIE2, but distinct from the mutation in the sporadic VM [28]. GVM in turn, is caused by a loss-of-function mutation in the glomulin gene, which is obviously an integral player in the regulation of angiogenesis, especially vascular smooth-muscle development [27,35,36].

### **2.2.3 Clinical presentations**

The estimated prevalence of VMs is approximately 1% [37,38]. As congenital, they are present at birth, but mostly become clinically apparent later in childhood [37,39]. They have a tendency to expand commensurate to the child's growth, but they never spread into new body areas or regress spontaneously. Hormonal stimulus during puberty or pregnancy typically aggravates their growth (Figure 5) [39].

Clinically, VMs are soft, compressible, and bluish when located superficially. Deeper lesions may manifest in asymmetry or pain, or may be discovered as incidental findings in imaging studies [40,41].

The most common symptoms are pain and swelling, often provoked by physical activity through enhanced circulation and distension. Local thrombosis in the malformation is a common cause of pain. VMs may also cause severe disfigurement, cosmetic harm, functional impairment, or systemic complications through blood coagulopathy [7,26,37,42].



**Figure 5.** A series of pictures demonstrating the commensurate and slow expansion of a leg VM. The bluish discoloration and superficial nodules become more visible over time, but do not invade new areas. The natural course of VM essentially differs from that of infantile hemangioma (Figure 3).

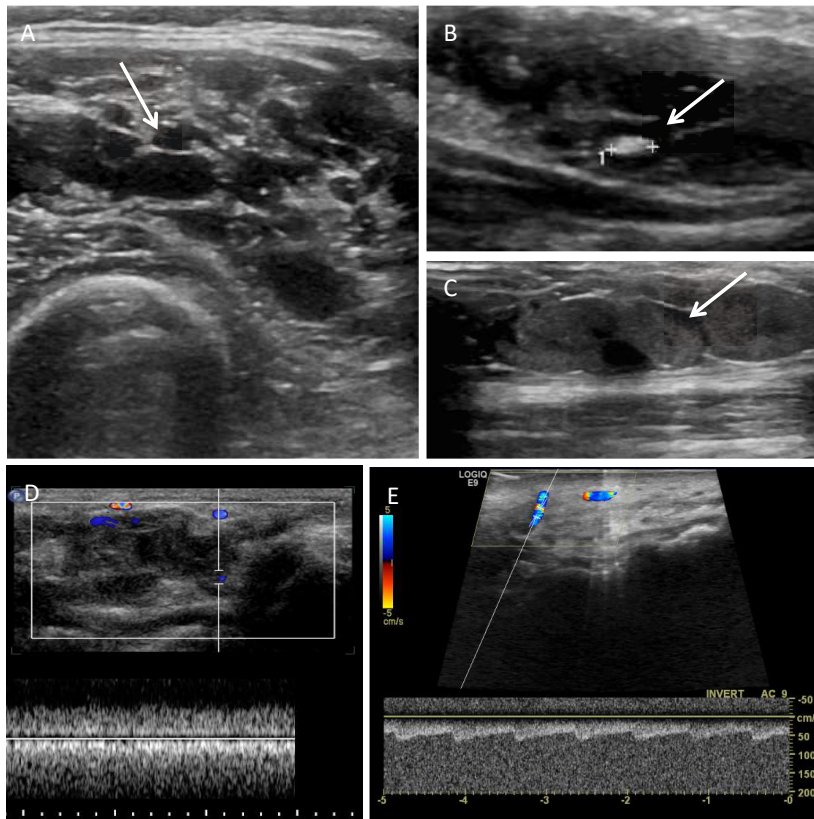
#### 2.2.4 Imaging

Imaging, especially ultrasonography (US) and magnetic resonance imaging (MRI), plays a central role in the evaluation of soft tissue lesions. US is often the first-line modality because of its good availability and usability without sedation even for small children [43]. In US, VMs appear as low-echogenicity, compressible, tubular structures that may invade subcutaneous fat or deeper tissues (Figure 6) [44]. The use of a tourniquet or standing position may facilitate their visualization as distension of the veins increases. The small field of view and restricted penetrance limits the applicability of US in extensive and deep lesions [24,43,45].

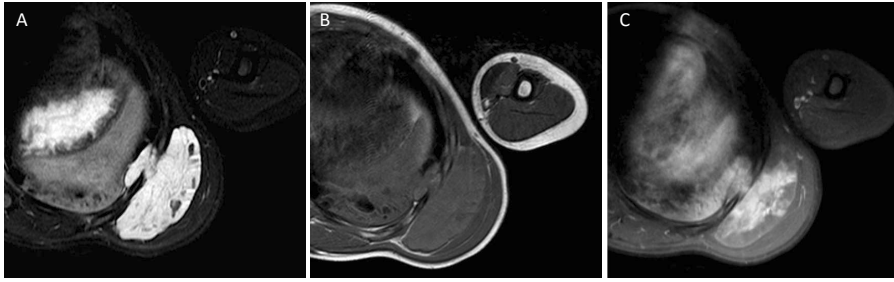
MRI complements the primary evaluation with US by elucidating the dimensions of the malformation, affected tissue planes, and relations to surrounding structures (Figure 7) [37,43,47].

A typical VM presents high signal intensity in T2-weighted and low to intermediate signal in T1-weighted sequences (Figure 7). It may consist of either densely packed, well-demarcated tubular structures or rather diffusely infiltrating phlebectasia [43]. The

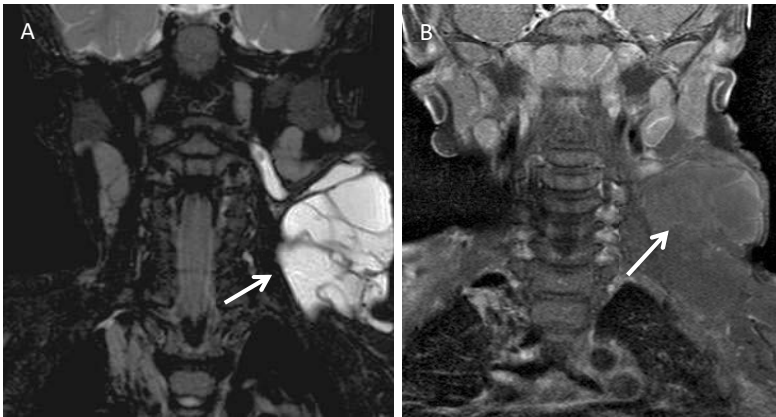
contrast enhancement varies from early and avid to partial and slow and differentiates VMs from lymphatic malformations, in which the vascular spaces do not enhance [43,48](Figure 8).



**Figure 6.** Characteristic US features of VMs. **A.** Intramuscular low-echogenicity tubular structures that are compressible upon pressing the US probe. **B.** Phleboliths emerge as small hyperechoic foci with shadowing. **C.** Thrombosis in the venous channels appears as heterogeneous echogenic mass, which reduces the compressibility of the vascular channels. **D.** The slow monophasic blood flow is mostly detectable with Color Doppler examination. Lack of detectable flow may be due to very slow velocity or thrombosis. **E.** Biphasic low-resistance flow indicates arteriovenous shunting not present in VMs.



**Figure 7.** Axial MRI of a VM in the left flank clearly delineates the width of the lesion and relations to surrounding anatomy. **A.** T2-weighted fat-saturated image shows a high signal intensity lesion consisting of cluster-like tubular structures and low signal intensity thrombi. **B.** The signal in T1 is isointense with or slightly higher than surrounding muscles. **C.** Post-contrast T1 image demonstrates partial enhancement of the vascular channels.



**Figure 8.** Coronal MRI of a neck lymphatic malformation. **A.** T2-weighted fat-saturated image shows the wide fluid-filled vascular spaces surrounded by thin walls and septa. **B.** T1-weighted fat-saturated post-contrast image demonstrates the lack of internal enhancement of the vascular spaces. Some faint delineation of the wall structures is, however, detectable.

Additional important differential diagnoses for VMs are the high-flow lesions, i.e. arteriovenous malformations (AVMs) and fistulas (AVFs). Their differentiation from VMs is crucial because of vastly different management strategies [1,49]. Flow void artifacts (Figure 9A) are characteristic for high-flow lesions and readily detectable in T1- and T2-weighted sequences [50]. Contrast-enhanced dynamic MRI (cedMRI) techniques reveal the flow dynamics in more detail and together with MRI have replaced conventional angiography in basic diagnostics (Figure 9B) [50-52].

Despite being slow-flow lesions, VMs commonly enhance readily at arterial or early venous phase, causing confusion among radiologists not familiar with vascular malformations. However, tortuous feeding arteries and early enhancing draining veins, characteristic for AVMs, are absent in VMs.

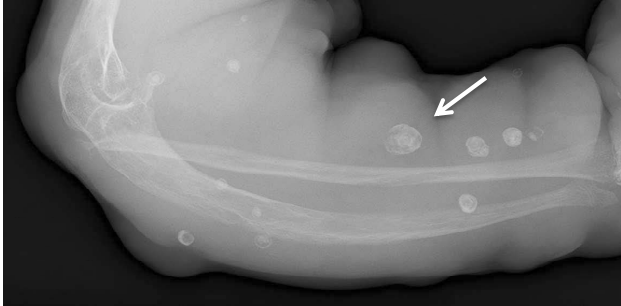


**Figure 9. A.** AVM of the hand with prominent flow voids indicating high-flow vessels. **B.** An arterial phase contrast-enhanced dynamic MRI (cedMRI) points out an AVM of the right thigh revealing the anomalous feeding arteries and early enhancing draining veins.

X-rays and computed tomography (CT), although revealing possible phleboliths, are mostly unnecessary in VM diagnostics (Figure 10) [53,54]. However, in rare cases of



bony involvement X-ray or CT may be helpful in the evaluation of the bone structure [37,43].

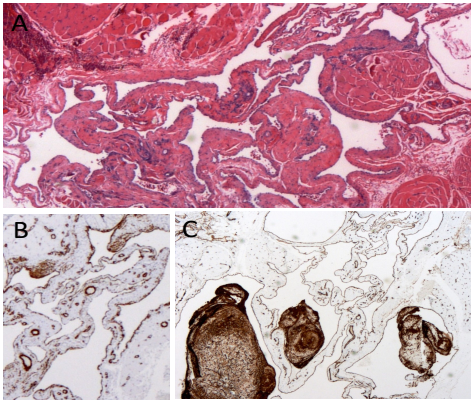


**Figure 10.** Phleboliths, readily visible in x-ray images, are calcified nodules originating from organizing thrombi in venous spaces. They are typical for VMs but are not present in all cases.

### 2.2.5 Histopathology

In typical cases, histology is not necessary for VM diagnosis. However, when the findings of patient history and clinical examination do not match imaging results, biopsy is mandatory to exclude malignancy and to achieve the correct diagnosis [45,55].

The histology of VMs consists of dilated and irregular venous-type channels that vary in size and form interconnecting networks among normal tissues (Figure 11) [12,56]. The wall structures of the anomalous veins are abnormally thin with a continuous flat endothelium but an irregular smooth muscle layer. Thrombosis is commonly present and it may organize to form fibromyxoid nodules or calcified phleboliths [57]. Specific immunohistochemical markers are used to reveal the abundant endothelium (CD31) and smooth muscle and pericytes (smooth-muscle actin (SMA)) (Figure 11). Podoplanin (D2-40), which is specific for lymphatic endothelium, stays negative in VMs, differentiating them from lymphatic malformations [12,58].



**Figure 11.** Typical histological features of VMs. **A.** Hematoxylin and Eosin staining of an intramuscular VM depicts the labyrinth-like interconnecting networks of venous channels. **B.** Smooth muscle actin staining demonstrates the irregularity of the smooth muscle layer in anomalous veins. **C.** CD31 staining highlights the endothelium of all vascular channels. The intraluminal thrombi also stain because CD31 is expressed in platelets in addition to endothelial cells.

### **2.2.6 Differential diagnostics of intramuscular vascular malformations; AST, FAVA, and PHOST**

Intramuscular VMs comprise a challenging group of lesions in terms of differential diagnostics and management [45,59-61]. These lesions often cause chronic pain and significant functional impairment. The response to sclerotherapy is variable, but often insufficient, necessitating repeated procedures and surgical resections. A growing body of evidence implies the presence of several distinct intramuscular vascular entities such as angiomatosis of soft tissue (AST), PTEN hamartoma of soft tissue (PHOST), and fibro-adipose vascular anomaly (FAVA). Because they present imaging features characteristic of slow-flow vascular malformations, they are easily mixed with VMs, but have specific histology and clinical behavior [45,59-62].

Rao and Weiss described already in 1992 a benign vascular lesion termed angiomatosis of soft tissue (AST) [62]. They delineated its histology as consisting of various size vessels, large veins with irregular walls, clusters of capillaries, and abundant

intramuscular fat. They assumed these lesions to be of generalized mesenchymal origin rather than pure vascular malformations and reported high recurrence rates after surgical resections [62]. Wassef described this same lesion in more histological detail in 2011 [61] and it has since been added to the new ISSVA classification (2014) under ‘provisionally unclassified vascular anomalies’ [5,61]. He pointed out the marked irregularities in the vessel walls; the smooth muscle in the dilated veins varies from thick to tenuous with sudden clefts, whereas the walls of the arteries are often hyperplastic, narrowing the lumens. Honeycomb-like thin-walled vascular spaces, disorganized smooth muscle, lymphatic vessels, and lymphocyte nodules are characteristically present as well [61].

PHOST, in turn, is a distinct type of intramuscular vascular lesion encountered in patients with PTEN hamartoma tumor syndrome (PHTS) [60]. Its histology closely resembles that of ASTs, also consisting of vessel proliferations of different calibers, clusters of capillaries, increased intramuscular fat, and fibrous tissue. However, PHOSTs may additionally include AV shunts, a feature not reported for ASTs [58,60].

Fibro-adipose vascular anomaly (FAVA), not described until 2014 by Alomari et al. [59], again shares many of the histological features characteristic for AST and PHOST. It is an intramuscular vascular lesion affecting primarily the lower limbs and is associated with muscle contractures and severe pain [59]. Alomari et al delineated the histology of 18 lesions, half of which were core needle specimens and the other half surgical specimens. The histological findings described were variable, including a dense fibro-fatty component, irregularly muscularized veins, lymphatic channels, thin-walled vascular spaces mimicking pulmonary alveoli, lymphocyte aggregates, thrombosis, and phleboliths. They did not report any AV shunts or abnormal arteries [59].

The MRI findings for common intramuscular VM, PHOST, and FAVA overlap [43,59,60], whereas those for AST are not previously described. VM, PHOST, and FAVA all appear as predominantly intramuscular lesions with high signal intensity tubular structures in T2 sequences. The T2 signal intensity is, however, typically more heterogeneous in FAVA and PHOST than in common VMs [59,60]. Intramuscular fat, appearing as high signal intensity areas in T1, is often especially abundant in PHOST

and FAVA, but may appear in common VM as well. Neither the T1 enhancement pattern after gadolinium nor the presence of phleboliths conclusively distinguishes these lesions from each other [45,59,60].

### **2.2.7 Blood coagulopathy**

VMs are associated with blood coagulation disorders in the form of elevated D-dimer in 33-62% of patients [63-66]. This condition, designated as localized intravascular coagulopathy (LIC), may be an incidental finding in screening laboratory analyses or manifest as local thrombosis and subsequent pain. Elevated D-dimer correlates with larger lesion sizes, visceral and muscle involvement, and the presence of phleboliths [64,67]. LIC also entails a risk of progression to disseminated intravascular coagulation (DIC) as a consequence of intrinsic or extrinsic stimulus such as any invasive procedure or infection [68]. In DIC, the fibrinogen is concomitantly decreased and prothrombin time may be prolonged due to increased consumption of coagulation factors [8,63,64,69-73]. LIC and its exacerbation predisposes patients to potentially severe thrombosis and bleeding complications [71,72,74]. The pathophysiology behind the coagulopathy remains somewhat unresolved. According to the theory of LIC, a constant coagulation and subsequent fibrinolysis takes place in the dilated venous channels, leading to excessive consumption of fibrinogen and other coagulation factors. The slow flow conditions, stagnation of blood, and abnormal endothelium are possible contributing factors [75]. However, the lack of systematic reports on fibrinolysis and coagulation activity, bleeding times, and thrombophilias in VM patients weakens these speculations.

The VM-related coagulopathy is different from the Kasabach-Merritt phenomenon, a condition encountered in association with rare pediatric vascular tumors such as kaposiform hemangioendothelioma and tufted angioma [76,77]. In Kasabach-Merritt phenomenon, platelets are trapped in the vascular tumor leading to potentially severe thrombocytopenia and bleeding diathesis, whereas in VM-related LIC the platelet count remains normal or only slightly decreased, but fibrin turnover and consumption of coagulation factors are increased. Thus, the etiology, treatment, and prognosis of these two conditions are completely different and they should not be confused.

The management of LIC in VM patients aims to reduce the VM size and blood stagnation [65,75]. The use of graded compression garments on extremity malformations diminishes the distention of the venous spaces, reducing swelling, pain, and functional impairment. It probably also alleviates the LIC, although this has not been systematically studied [65,75]. Invasive procedures, such as sclerotherapy, surgery, and laser ablation, all aim to reduce the mass of the malformation, but conversely may serve as triggers for exacerbation of LIC [78]. Anticoagulation therapy in the form of low-molecular-weight heparin (LMWH) has proven effective in diminishing thrombosis and pain episodes and in normalizing D-dimer and fibrinogen levels [63,76].

### **2.2.8 Treatment**

VMs require individually tailored treatment strategies because of the highly variable clinical picture. An interdisciplinary approach in the form of regular patient meetings is pivotal in achieving proper diagnostics and optimal treatment for each patient [79,80]. Since curative management is rarely available, the aim is to alleviate symptoms and relieve cosmetic disfigurement. The current main treatment options for VMs include conservative treatment, sclerotherapy, surgery, laser ablation, and pharmacological treatment [25,81,82]. The literature on treatment outcomes lacks uniform and reliable methods for the evaluation of subjective symptoms and functional impairment, hindering the comparison between different modalities [14,83-90].

#### **2.2.8.1 Conservative treatment**

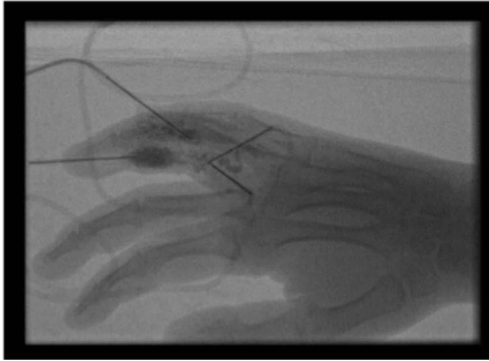
After proper diagnostics and counseling, asymptomatic VM patients do not necessarily need any treatment. Children and adolescents should, however, be followed up for the progression tendency of VMs [10,39]. Graded compression garments are suitable as the only treatment for extremity VMs with mild symptoms, or combined with any invasive treatment for more symptomatic patients [37,49,82,91]. They seem to alleviate symptoms and functional impairment, probably by reducing blood volume and distension of the malformation and forcing the blood into the deep venous system. The garments must be individually tailored to provide optimal support and used preferably during the daytime or at least when physically active.

### **2.2.8.2 Sclerotherapy**

Sclerotherapy is currently the primary treatment for most symptomatic VMs [19,25,92,93]. It is less invasive than surgery and also applicable for lesions considered non-resectable. On the other hand, repeated sessions are commonly required to achieve satisfactory response [19,94].

The aim of sclerotherapy is to shrink and obliterate the malformation by permanently closing the anomalous vessels. The locally injected sclerosing agent induces endothelial cell damage followed by inflammatory response, fibroblast proliferation, and sclerosis [95]. The mechanism of action depends on the type of sclerosant used and is not exactly understood. The influence may be chemical, physical, and/or biologic [95]. Thrombosis, cell dehydration by osmosis, and extraction of proteins from lipids are contributing factors in the disruption of biologic function of the targeted endothelium [95]. Understanding the nature of the malformation, properties of the sclerosants in use, delivery methods, optimal concentrations, effects of flow, and potential side effects is essential for success of the treatment.

The sclerosing agent is injected percutaneously into the vessel lumens using US and fluoroscopy guidance. A prior contrast injection is necessary to estimate the volume of the lesion and to determine the routes of the draining veins. Intravenous access is confirmed by venous back-flow from the inserted needle. A tourniquet or manual compression of the draining veins minimizes the risk for systemic escape of the sclerosant. Usage of the draining technique, in which two or several needles are placed in different parts of the malformation, one for injecting the sclerosant and the others for draining the blood and excessive sclerosant, helps to avoid overfilling the lesion and ensures flushing the whole VM with the sclerosant (Figure 12) [19,37].



**Figure 12.** Fluoroscopy image of a sclerotherapy procedure with the draining technique. Several needles are placed in the VM to ensure the escape of excessive sclerosant and to prevent overfilling of the lesion.

The most commonly used sclerosing agents for VMs include ethanol, sodium tetradecyl sulphate (STS), polidocanol, and bleomycin. They are all applicable and differ mainly by their effectiveness and risk profiles [14,19,84,96-98]. Ethanol is the most effective sclerosant due to its high toxicity and ability to cause instant endothelial damage. Its usage has diminished because of the common adverse effects, such as postprocedural pain and swelling as well as the high complication rates [19,88,99-104]. The detergent sclerosants, i.e. STS and polidocanol, are less potent but more versatile due to their capacity to form foam and their availability in different concentrations. The detergents induce less pain upon injection and the risk for complications is lower than with ethanol [105,106]. Bleomycin is commonly used for lymphatic malformations, but is applicable also for VMs. It is an antibiotic derivate, originally approved for oncologic use for its cytotoxic properties. Bleomycin has a lesser tendency to cause swelling and is thus especially suitable for anatomically confined and delicate locations [88]. Ethylcellulose-ethanol is a newly developed agent in which ethanol is combined with rapidly forming ethylcellulose gel. This gel framework prevents ethanol from escaping the target vessel thereby increasing the local influence to the endothelium and diminishing adverse effects [107] [107].

### **2.2.8.3 Additional endovascular techniques**

Additional endovascular techniques, such as gluing and coiling, can be combined with percutaneous sclerotherapy in selected cases [19]. Closing large draining veins with intravascular coils is practical when manual compression or use of tourniquet is not sufficient. Glue may be used for closing especially wide venous spaces or anomalous truncular veins.

### **2.2.8.4 Sclerotherapy complications**

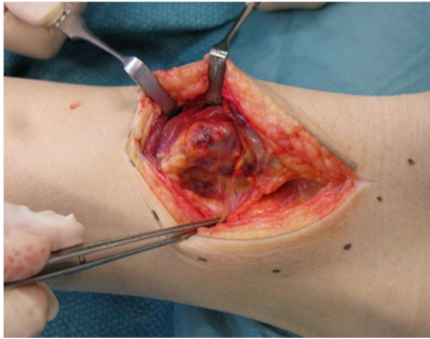
Despite the mini-invasive nature of sclerotherapy, both local and systemic complications may occur. The most common complications include local skin blistering, intensification of pain and swelling, neural paresthesia, and skin discoloration [83,92,94,108-115]. Possible systemic complications include hemoglobinuria, affecting up to 34% of patients after sclerotherapy, and accompanied by transient oliguria in 57% of cases[116]. Paradoxical gas embolism and subsequent stroke is a very rare but potential complication of foam sclerotherapy [117,118]. A right-to-left heart shunt, especially patent foramen ovale, is the most common predisposing factor [118]. The risk for paradoxical embolism can be diminished by preparing the foam with CO<sub>2</sub> instead of air. Single case reports cover extensive muscle necrosis and infection following ethanol and STS sclerotherapy [99], pulmonary embolism [119], and reversible and fatal cardiovascular collapse after polidocanol and ethanol sclerotherapy, respectively [100,120]. In each individual case, the complication risk must be weighed against the expected benefits and discussed with the patient beforehand.

### **2.2.8.5 Surgery**

Since the development of sclerotherapy techniques, the role of surgery has diminished. It still, however, remains an important modality for selected cases [86,121]. For example, completely clotted VMs with persisting symptoms, symptomatic glomuvenous malformations, intramuscular FAVA lesions not responding to conservative treatment, and well-demarcated VMs of the palm and hand may benefit from primary surgical excision. Moreover, patients with intra-articular VMs, especially affecting the knee joint, benefit from surgical treatment [122]. Recurrent hemarthroses and subsequent cartilage damage can be avoided by early synovectomy. In addition,



patients with poor response to sclerotherapy may benefit from secondary surgical resections (Figure 13) [86]. Adjunctive sclerotherapy is considered applicable in cases of large malformations to reduce their size and extent before resection [123,124]. Preoperative glue embolization with percutaneously injected n-butyl cyanoacrylate (n-BCA) has been used successfully for the treatment of facial VMs [125]



**Figure 13.** Surgical excision of an intramuscular VM of the soleus muscle. The surgical strategies for VMs must be highly individualized and carefully planned with detailed imaging. Image courtesy of Erkki Tukiainen/Pia Vuola, HUH, Department of Plastic Surgery.

#### **2.2.8.6 Laser therapy**

Laser ablation has gained ground in the treatment of saphenous vein insufficiency and tributary varices [126]. It has since been applied for the treatment of infantile hemangiomas and vascular malformations, with promising results especially for VMs [14,90,127-130]. Several types of laser applications are in use, diode, CO<sub>2</sub>, and nd:YAG laser being the most common. The aim of laser therapy is to cause thermal damage directly to the endothelium, leading to vessel closure by fibrotic obliteration [126]. Laser ablation is regarded as mini-invasive without systemic influences. The most common complications include local skin blisters, pain, ecchymosis, and paresthesia [126]. Use of local tumescent anesthesia during the procedure diminishes the risk for complications [131]. Perivenously injected tumescent anesthesia reduces

pain, protects surrounding tissues by cooling and insulating, and increases contact area between laser tip and endothelium through vasoconstriction [131,132]. In a series of 32 patients with upper aerodigestive tract VMs, 980 nm diode endovenous laser proved effective in terms of reducing dysphagia and obstructive sleep apnea [90]. In another series of 164 peripheral VMs, the clinical success rates after endovascular diode laser treatment were 98% for reduction of pain and 69% for cosmetic outcome [126].

#### **2.2.8.7 Medical therapy**

Limited case series have described the efficacy of the mammalian target of rapamycin (mTOR) inhibitor, sirolimus, in the treatment of complicated vascular anomalies [133-135]. The rationale behind these promising results is the key role of mTOR in the regulation of angiogenesis and development of vascular anomalies [33,136]. Sirolimus has proved especially promising in the treatment of complex lymphatic anomalies and lymphatic malformations as well as combined lymphatic-venous malformations [134]. Ongoing clinical trials will soon provide more data on the role of sirolimus in the management of complex venous malformations refractory to other treatments.

### **3. Aims of the study**

This thesis aimed to study histology and imaging in differential diagnostics of intramuscular VMs, blood coagulation disorders in pediatric VM patients, and safety aspects of sclerotherapy treatment for VMs. Specifically, the objectives of Studies I-IV were to investigate the following:

- 1) Whether different histological entities are identifiable in the histological specimens of VMs operated on for poor sclerotherapy response, and whether they have any specific preoperative imaging findings (I).
- 2) What specific findings children with VMs have in extended analysis of blood coagulation and fibrinolysis activity in the quiescent clinical phase, and how these correlate with VM's clinical and imaging features (II).
- 3) The complication rate of sclerotherapy for trunk and extremity VMs (III) and head and neck VMs (IV), together with possible predisposing factors for and measures to avoid sclerotherapy complications (III, IV).

## **4. Materials and methods**

### **4.1 Summary of methods (I-IV)**

#### **4.1.1 Study design and ethical considerations**

This thesis was prepared in cooperation with the interdisciplinary team for vascular anomalies at Helsinki University Hospital (HUH). The team coordinates diagnostics, treatment, and follow-up for vascular anomaly patients in the HUH district of ca. 1.5 million people as well as for referral patients from other university hospital districts in Finland. The team consists of specialists in interventional and pediatric radiology, neuroradiology, pediatric, plastic, vascular, and maxillofacial surgery, otorhinolaryngology, pathology, dermatology, ophthalmology, and hematology. In addition to the regular patient meetings, the team has an active scientific input.

The institutional review board of HUH approved the study protocol. The study design was retrospective utilizing of patient records, imaging, and histological specimens.

#### **4.1.2 Patients**

The study populations in Studies I, III, and IV consisted of consecutive VM patients treated with sclerotherapy in HUH from 1 January 2007 to 31 August 2013. Study I included patients with extremity VMs (n=102), Study III patients with trunk and extremity VMs (n=127), and Study IV patients with head and neck VMs (n=75) (Table 1). The HUH interdisciplinary team for vascular anomalies had evaluated the patients and recommended the treatment. The diagnosis of each patient was additionally confirmed from imaging studies (MRI, US, and periprocedural phlebography) according to the ISSVA classification (1996).

The study population in Study II (n=62) was collected from the outpatient registry of the pediatric surgery clinic at Helsinki Children's Hospital from 1 January 2002 to 31 December 2015 (Table 1). Only patients with VMs and available complete laboratory results of blood coagulation activity, bleeding tendency, and blood cell count from one clinically stable time point were included.

**Table 1.** Study populations in Studies I-IV.

	<b>N (screened)</b>	<b>Age median</b>	<b>Male/Female</b>	<b>VM location</b>
<b>Study I</b>	19 (102)	21	40/62	extremity
<b>Study II</b>	62	12	27/35	all
<b>Study III</b>	127	22	54/73	trunk, extremity
<b>Study IV</b>	75	33	24/51	head, neck

VM, venous malformation

#### 4.1.3 Methods

In Study I, we evaluated the clinical records of 102 consecutive patients treated with sclerotherapy for extremity VM and identified those who had later been operated on for insufficient or poor sclerotherapy response (n=19). We analyzed the histological specimens of the operated patients with a semi-quantitative grading scale and concluded the histological diagnoses accordingly (Table 2). Finally, we qualitatively compared the histological diagnoses with the preoperative MR imaging by analyzing the morphology of the malformation, amount of fat tissue visible in T1-weighted sequences, presence of phleboliths, and enhancement patterns after gadolinium.

**Table 2.** Histological features and their associations with different intramuscular vascular anomalies [17,59,61].

<b>Histological features</b>	<b>VM</b>	<b>AST</b>	<b>FAVA</b>
Wide venous spaces	+++	++	+++
Honeycomb-like vessels	-	++	+++
Lymphocyte clusters	-	+++	+
Thick-walled artery-like vessels	-	++	-
Intramuscular fat	+	+++	+++
Fibrosis	+	+++	+++
Disorganized smooth muscle	-	+++	-
Phleboliths	++	+	++
Small vessel component	+	+++	+

VM, venous malformation; AST, angiomatosis of soft tissue; FAVA, fibro-adipose vascular anomaly

In Study II, we systematically analyzed the laboratory results, including coagulation and fibrinolysis activity, coagulation times, thrombophilia markers, and blood cell counts, of 62 pediatric VM patients. All laboratory results were from one clinically stable time point. We analyzed correlations between abnormal laboratory variables and clinical and imaging findings of the patients and malformations, derived from patient records and MRI studies. We additionally recorded any anticoagulation therapies the patients had undergone, and all coagulopathy-related complications.

In Studies III and IV, we retrospectively analyzed sclerotherapy-related complications of 202 VM patients undergoing a total of 280 peripheral and 150 head and neck sclerotherapy procedures. We graded the complications according to the Clavien–Dindo complication classification (Table 3). It is based on the management necessitated by complications, and has previously only been used for the reporting of surgical complications. All post-procedural disabilities, except for transient pain or swelling lasting less than two weeks, were considered complications. Insufficient symptom relief was not regarded as a complication. We analyzed whether complications were associated with VM features (location and involved tissue planes) or treatment properties (the sclerosant used, number of treatments, or history of previous surgery) of peripheral (III) and head and neck (IV) VMs.

**Table 3.** Summary of the Clavien–Dindo classification for complications.

Grade	Definition
I	Conservatively manageable complications without need for pharmacological treatment or surgical, endoscopic, or radiological interventions. Acceptable therapeutic regimens are: drugs as antiemetics, antipyretics, analgetics, diuretics, electrolytes, and physiotherapy.
II	Necessitating pharmacological treatment with drugs other than acceptable for grade I
III	Necessitating surgical, endoscopic, or radiological intervention
IIIa	Without general anesthesia
IIIb	With general anesthesia
IV	Life-threatening complication requiring intermediate care/intensive care-unit management
IVa	Single-organ dysfunction (incl. dialysis)
IVb	Multiorgan dysfunction
V	Death of patient

#### **4.1.4 Statistics**

Statistical analyses were conducted with Microsoft Excel 2011 and NCSS 8 statistical software. Chi-Square test or Fisher's exact test and Mann-Whitney U-test served for paired comparisons. For correlation calculations we used Pearson's correlation analysis and Spearman's rank (non-parametric variables). We considered p-values  $< 0.05$  to be statistically significant.

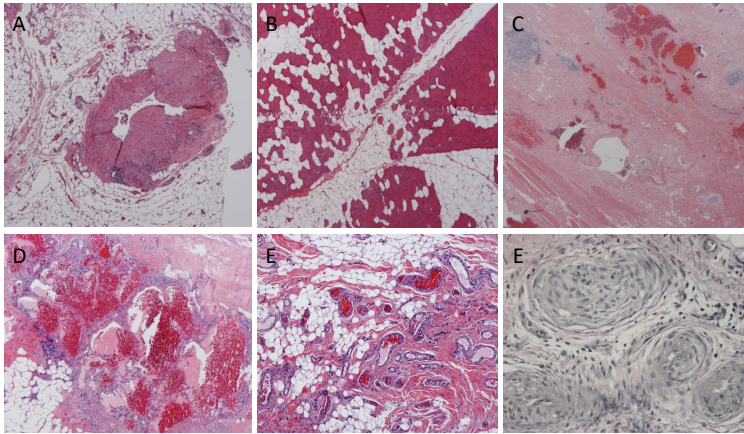
## **5. Results**

### **5.1 Angiomatosis of soft tissue is an important differential diagnosis for intramuscular venous malformations (I)**

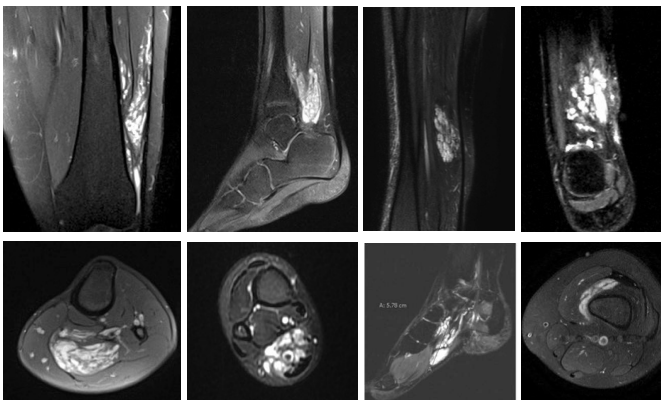
Of the 19 patients operated on for insufficient sclerotherapy response, 15 had an intramuscular lower extremity malformation. The histological diagnosis for 13/15 intramuscular lower extremity lesions was angiomatosis of soft tissue (AST) or fibro-adipose vascular anomaly (FAVA), while the remaining six operated lesions were compatible with common VMs (Table 2). AST and FAVA were not clearly distinguishable from each other based on histological picture (Table 2). Figure 14 shows the most important histological features for AST.

The MRI findings for common intramuscular VMs and ASTs were overlapping; both emerged as tubular structures with signal high in T2 and low to intermediate in T1 sequences (Figure 15). The internal enhancement after gadolinium and amount of intramuscular fat was variable in both. Phleboliths, commonly present in VMs, were occasionally present in ASTs as well. Fluid-fluid levels and cluster-like appearance were more characteristic for VMs, whereas heterogeneous T2 signal, visibility of small vessels in addition to dilated veins, and diffuse enhancement implied AST.





**Figure 14.** Histological features for AST. **A.** A wide venous space with markedly thick and irregular smooth muscle wall surrounded by intramuscular fat. **B.** Mature fat interspersed among muscle fibers **C.** Lymphocyte clusters, honeycomb-like vascular spaces, and fibrosis. **D.** Honeycomb-like vascular spaces filled here with erythrocytes, but commonly stained positive with podoplanin, specific for lymphatic endothelium. **E.** Clusters of small vessels. **F.** Elastic staining of thick-walled artery-like vessels demonstrating lack of elastin layer, characteristic for normal arteries, in their walls.



**Figure 15.** T2-weighted fat-saturated MR images of different lower extremity AST lesions demonstrating overlapping imaging features with intramuscular VMs.

## 5.2 Venous malformations are associated with diverse abnormalities in the blood coagulation system in pediatric patients (II)

Among the 62 evaluated pediatric VM patients overt DIC or thrombocytopenia did not occur and fibrinogen was normal in all but one patient. The prevalence of congenital thrombophilias did not differ from the general population. Substantial abnormalities were, however, detectable in leukocyte, antithrombin, coagulation factor (FVII, FVIII, and FXIII), and D-dimer levels (Table 4). D-dimer was elevated in 39%, indicating enhanced fibrin turnover. FVII and FXIII were decreased, whereas FVIII and antithrombin were elevated in one-fifth of patients. The elevation of D-dimer ( $p=0.07$ ) and decrease of FXIII ( $p=0.03$ ) were most common in the largest lesions ( $> 25$  cm), in lesions spreading into multiple locations ( $p=0.01$ ), and in cases with diffuse morphology (D-dimer  $p=0.02$ , FXIII  $p=0.21$ ). FVIII, instead, was never elevated in the largest lesions or in lesions with deep locations. FVIII elevation was associated with discrete morphology ( $p=0.001$ ) and less reported pain at time of initial evaluation ( $p=0.02$ ). Antithrombin (elevated in 55%) and leukocytes (decreased in 33%) did not correlate with any of the clinical findings.

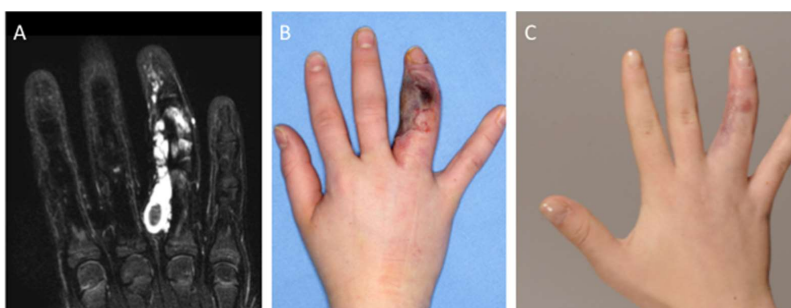
**Table 4.** Coagulation profiles of the pediatric VM patients.

Coagulation marker	Reference range	N	Median	Range	Values below the reference limit N (%)	Values above the reference limit N (%)
Leukocytes (E9/L)	5-14	57	5.8	2.8-14.9	<b>19 (33.3)</b>	1 (1.8)
Platelets (E9/L)	200-450	58	267.0	172-619	6 (10.3)	1 (1.7)
Fibrinogen (g/L)	1.7-4	60	2.9	0.0-5.2	1 (1.7)	6 (10.0)
Prothrombin time (%)	70-130	62	94.5	66-157	4 (6.6)	4 (6.5)
APTT (s)	24-40	61	27.0	24-49	0	2 (3.3)
Thrombin time (s)	17-25	62	18.0	15-37	6 (9.7)	1 (1.6)
Antithrombin (%)	84-108	62	110.0	84-134	0	<b>34 (54.8)</b>
FV (%)	79-128	61	102.0	56-136	6 (9.8)	2 (3.3)
FVII (%)	76-170	59	90.0	48-222	<b>12 (20.3)</b>	1 (1.7)
FVIII (%)	52-148	62	119.5	75-206	0	<b>11 (17.7)</b>
FXIII (%)	76-156	60	95.5	53-148	<b>12 (20.0)</b>	0
Protein C (%)	74-141	61	91.0	65-173	3 (4.9)	3 (4.9)
Protein S Ag (%)	50-137	62	100.0	42-143	1 (1.6)	3 (4.8)
vWFRCo (%)	44-183	53	82.0	44-220	0	2 (3.8)
vWF-Ag (%)	51-169	58	89.5	50-187	1 (1.7)	1 (1.7)
D-dimer (mg/L)	< 0.5	61	0.3	0.1-15	0	<b>24 (39.3)</b>

### 5.3 Superficial lesion location, use of ethanol, and blood coagulopathy are associated with increased risk for sclerotherapy complications (III, IV)

#### 5.3.1 Sclerotherapy complications for trunk and extremity venous malformations (III)

Complications occurred in 35 procedures for 31 patients, the complication rate per procedure being 12.5%. Most of the complications (83.3%) were local and conservatively manageable grade I-II complications, of which local skin damage and prolonged pain or swelling were the most common. Two skin wounds were managed with skin grafting, compatible with grade III complications (Figure 16). In addition, four severe complications occurred: three grade IV and one fatal grade V complication. All patients suffering severe complications had blood coagulopathy with elevated D-dimer and decreased fibrinogen level. Predisposing factors for local complications were superficial lesion location ( $p=0.049$ ) and use of ethanol as a sclerosant ( $p=0.02$ ). Use of STS vs. polidocanol, lesion location (extremity vs. trunk), previous surgery, number of procedures, or patient age were not associated with the occurrence of complications.



**Figure 16.** A local complication after sclerotherapy with sodium tetradecyl sulphate 3% for a previously operated finger VM. **A.** T2-weighted fat-saturated MRI shows the subcutaneous malformation with intra-osseal component. **B.** Sclerosant's local toxicity resulted in deep skin necrosis that was classified as a grade III complication due to need for surgical management. **C.** Three months after skin grafting.

### **5.3.2 Sclerotherapy complications for head and neck venous malformations (IV)**

Among the 75 head and neck VM patients who underwent 150 sclerotherapy procedures, the overall number of complications was 15, suffered by 13 patients. The complication rate per procedure was 10.0%. Twelve complications were treated conservatively (grade I and II), while three complications necessitated extensive and prolonged postprocedural management (grade III and IV). The patients with complications had a higher number of sclerotherapy procedures ( $p=0.009$ ) and more commonly surgical treatment after sclerotherapy ( $p=0.007$ ). The head and neck complications were not associated with the lesion location or depth or with the sclerosant used. Complications occurred, however, proportionally more often with bleomycin and when combining ethanol with other sclerosants.

### **5.3.3 Severe sclerotherapy complications (III, IV)**

Although complications were, in most cases, local and healed with conservative management, severe complications, requiring extensive postprocedural management, occurred in seven sclerotherapy sessions (1.6%). The malformations in patients with severe complications were extensive or had challenging locations. The trunk or extremity VMs were additionally complicated by blood coagulopathy. The mechanisms of severe complications remained partly obscure and these complications occurred despite careful preprocedural planning and treatment of the coagulation disorder in close cooperation with hematologists. The severe complications included:

- Massive intra-abdominal bleeding resulting from sclerotherapy for extensive retroperitoneal VM. This complication was treated with intravascular embolization of the left phrenic artery, blood transfusions, and stabilization at the intensive care unit (III).
- Two episodes of bleeding and infection of resulting hematoma, complicated by worsening of blood coagulopathy. These complications followed two separate procedures of sclerotherapy, laser, and gluing for an extensive pelvic and perirectal malformation. They were treated with intravenous antibiotics, blood and coagulation factor transfusions, and prolonged hospitalization (III).

- Fatal intracerebral bleeding after sclerotherapy for an extremely wide upper body malformation with severe coagulopathy (III).
- Necrosis of maxilla and three teeth resulting from STS sclerotherapy for palate VM. This complication required surgical removal of necrotic tissue and caused permanent bone defect, allodynia, and infraorbital nerve defect (IV).
- Extensive skin and muscle necrosis resulting from STS sclerotherapy for a frontotemporal VM. This complication required several surgical revisions, skin grafting and scar correction and caused permanent scarring and loss of function of the right frontal muscle (IV).
- Extensive intraprocedural bleeding of a tongue VM managed with particle embolization, resulting in partial tongue muscle necrosis and infection. This complication treated with intravenous antibiotics and partial resection of the tongue resulted in permanent dysfunction and articulation problems (IV).

## **6. Discussion**

This thesis focuses on three different aspects of VMs: differential diagnostics of intramuscular VMs (I), blood coagulation in pediatric VM patients (II), and sclerotherapy safety for trunk and extremity (III) and head and neck (IV) VMs.

AST was the predominant histological entity among intramuscular VMs operated on for unsatisfactory sclerotherapy response (I). Pediatric VM patients had diverse abnormalities in hemostatic markers that were associated with clinical and imaging findings of the VMs (II). The vast majority of sclerotherapy complications were local and conservatively manageable (III, IV). Local complications were associated with superficial lesion location and use of ethanol as a sclerosant (III). Blood coagulopathy was an important predisposing factor for the few severe complications (III).

### **6.1 Differential diagnostics and selection of treatment for intramuscular venous malformations (I)**

Even though the differential diagnostics between VMs and other vascular anomalies is established in general, novel challenges emerge with the newly recognized vascular entities such as angiomatosis of soft tissue (AST), fibro-adipose vascular anomaly (FAVA), and PTEN hamartoma of soft tissue (PHOST). Distinction of these entities from common VMs seems to have clinical relevance for the differences in optimal treatment strategies [59-62]. While sclerotherapy is applicable for most VMs, it is obviously less effective for AST, PHOST, and FAVA because of their solid fibro-fatty tissue and vascular components not responsive to sclerosants [60,61].

According to our study, histological features consistent with AST prevailed among lower extremity intramuscular lesions that were first diagnosed as VMs but later operated on for insufficient sclerotherapy response (I). These histological features, however, also closely resembled those previously described for FAVA and PHOST [59,60]. As FAVA and PHOST have both been described with limited patient numbers and in selected clinical contexts, they possibly all represent histologically the same entity with varying clinical manifestations. We termed these lesions as ASTs since AST

is the first designation in the literature for this histological entity and its definition is without specific descriptions of the clinical picture [62]. AST is also added to the current ISSVA classification under ‘provisionally unclassified vascular anomalies’ [5].

Because we only had histological specimens of the surgically treated malformations that had not responded to sclerotherapy, the number of AST lesions treated successfully with sclerotherapy remains unknown. Therefore, definite conclusions about the optimal treatment for AST are not justified based on our study. Regarding the histological composition of AST and the high proportion of ASTs among operated lesions, our findings do, however, imply that sclerotherapy is not the primary treatment for these lesions (I). Even so, sclerotherapy may reduce the volume of AST lesions with prominent venous component, thereby alleviating the intramuscular pressure and distension. On the other hand, as very few common VMs necessitated secondary surgery, sclerotherapy seems to be effective for common VMs also in the intramuscular location (I).

For these reasons, the pretreatment differentiation between common VMs and ASTs is important. As we could not identify specific MRI findings for AST, we emphasize the role of histology in differential diagnostics (I). Obtaining adequate core needle biopsies from VMs and ASTs has, however, proved challenging as the specimen often impinges on a vessel wall and becomes fragmented and scarce. According to our experience, US may be helpful in differential diagnostics, revealing the small arteries of AST and demonstrating less compressibility with AST than with common VM. This has, however, not been systematically studied.

## **6.2 Venous malformations and blood coagulation (II)**

Based on clinical observations and previous studies, the association between blood coagulation disorders and VMs is convincing. Several studies report elevated D-dimer and decreased fibrinogen in VM patients [8,64,67,69,76,137]. Blood coagulopathy also predisposes VM patients to severe complications, especially during invasive procedures [71,72,78].

Unlike the previous studies concentrating mainly on D-dimer and fibrinogen, we extended the analysis to cover detailed laboratory analysis of both coagulation and fibrinolysis activity as well as blood cell counts (BCC) (II). This enabled us to detect new and more diverse VM-related abnormalities in blood coagulation. Moreover, our unique study population comprising only children and timing of the blood sampling during a stable clinical phase presumably facilitated observation of VM-specific findings (II).

D-dimer was elevated in 39% of our pediatric patients, compatible with previous studies [67,69], whereas overt DIC with hypofibrinogenemia and thrombocytopenia did not appear (II). Significant abnormalities appeared, however, in leukocyte (low), antithrombin (high), FVII (low), FVIII (high), and FXIII (low) levels. Moreover, FVIII, FXIII, and D-dimer were associated with clinical features of the malformations.

The prevailing theory, designated as localized intravascular coagulation (LIC), explains the VM-related coagulopathy by constantly occurring local coagulation, excessive consumption of coagulation factors, and subsequently enhanced fibrin turnover [8,75]. This theory, not applied for any other conditions, is based on findings in the peripheral blood and established without local blood sampling or detailed evaluation of the coagulation biomarkers.

Our findings are not explained by the theory of LIC; the deficiencies in FVII and FXIII activities did not correlate with the elevation of D-dimer, decrease of fibrinogen, or other findings of enhanced consumption. Furthermore, unlike in conditions with enhanced consumption, antithrombin and FVIII were commonly elevated. Although the general significance of elevated antithrombin is unclear, we assume that it protects VM patients against excessive coagulation activity. High FVIII level has previously been linked to increased risk for venous thrombosis [138]. However, in our study, patients with elevated FVIII had less local pain and thus presumably less thrombosis. FXIII, in turn, has a central role in the stabilization of fibrin clot [139]. Decreased FXIII activity, as in one-fifth of our patients, makes the clot more loose and susceptible to fibrinolysis, thereby increasing the fibrin turnover. Moreover, a recent study shows that specific effects of the VM causative TIE2 mutation lead to activation of the



plasminogen/plasmin protease system, also contributing to fibrin degradation in VM patients [30].

A growing body of evidence indicates close interrelation between coagulation and angiogenesis [30,33,140,141]. Also in our study, many of the coagulation biomarkers associated with VMs are active contributors to the regulation of vessel development and maintenance [30,140-142]. Better understanding of the mechanisms behind this co-regulation will elucidate the pathophysiology of VMs and related coagulopathy.

### **6.3 Sclerotherapy safety for venous malformations (III, IV)**

Regarded as mini-invasive and generally safe, sclerotherapy has become an essential treatment modality for VMs during the past decade. Systematic evaluation of complications of any treatment is, however, essential to improve the safety of the process and to gain tools for critical comparison of available treatment options. However, the definition of complication markedly differs between studies, hampering their comparison. For example, many studies do not report self-healing superficial skin blisters as complications or only report ‘major’ complications. Moreover, varying complication classifications, study populations, sclerotherapy procedures, and sclerosants make the complication rates uncomparable [83,94,97,115,143].

To gain a more complete picture, we defined complication as any disability developing as a consequence of the treatment, including small skin wounds (III, IV). We also introduced the Clavien–Dindo classification (Table 2) [144,145] for the evaluation of sclerotherapy complications in order to improve accuracy and facilitate comparison with other reports (III, IV). Based on the management needed for the complication, the Clavien–Dindo classification is more objective and informative than the subjective division of complications into minor and major.

We consider our complication rates, 10.0% for head and neck and 12.5% for trunk and extremity VMs, to be acceptable. Majority of the complications were local grade I-II complications, neither influencing treatment outcome nor patient satisfaction in most cases (III, IV). Grade I-II complications are often avoidable by careful sclerotherapy

technique and selection of appropriate sclerosant. Awareness of potentially very severe complications is important, especially when treating very extensive VMs or VMs in critical locations. Such complications occurred in 1.6% of the sclerotherapy procedures, blood coagulopathy being a significant predisposing factor. Despite careful preparation and skilled management, complications do occur. Interdisciplinary cooperation is pivotal not only in diagnostics and treatment planning but also in managing complications.

Superficial lesion location predisposed to skin damage through the local toxicity of the sclerosant. This risk can be diminished, although not totally effaced, by using detergents with low concentrations. The efficacy of a sclerosant, however, more or less parallels its toxicity. Ethanol, for example, is the most potent sclerosant, but predisposes to both local tissue damage and severe systemic complications [99,101,102]. The use of ethanol was associated with the occurrence of complications also in our study, although utilized only in a limited number of cases at the beginning of the study period (IV). Ethylcellulose-ethanol is a new sclerosant with promising preliminary results [107,146]. It combines the efficacy of ethanol with a lower tendency to drain off the target place, thereby causing less complications.

One important finding was the association between gluing and infections (IV). Glue was applied in a limited number of procedures in order to close wide venous spaces in which sclerotherapy alone was ineffective. As a foreign material it, however, offers together with stagnant blood a propitious bed for bacterial growth. We have since started to use antibiotic prophylaxis when combining gluing with VM sclerotherapy.

Considering the variability of symptoms and disabilities caused by VMs as well as the individual opinion and experience of each patient, general treatment strategies applicable to all VMs are impossible to establish. The treatment options must be carefully discussed with each patient and the complication risk weighed against the subjective symptoms and expected benefit from the treatment.

## **6.4 Potential limitations of the study (I-IV)**

This study was limited by the retrospective study design. Any measurement of subjective symptoms and disabilities was difficult based on the patient records only, not allowing us to reliably report the sclerotherapy outcomes (I,III,IV). We hence used the need for secondary surgery as an end variable (I). The patient number was relatively low, especially for the comparison of the safety of different sclerosants (III, IV) and hemostatic variables in different diagnostic subgroups (II). The sclerotherapy setting is highly variable depending on the individual features of each patient and malformation. Consequently, the treatment outcome and complication risk are influenced by numerous unstandardized factors in addition to those analyzed here (III, IV).

## **6.5 Future prospects**

This research raised many further questions to be answered:

- Although now better able to differentiate ASTs from common VMs, the optimal treatment option for these often very symptomatic patients remains unclear. Prospective evaluations of long-term outcomes of surgical treatment would be valuable.
- As AST lesions closely resemble PHOSTs by histology, genetic studies evaluating PTEN mutations both locally from AST lesions and systemically from the blood of these patients, could help to establish the connection between AST and PHOST.
- According to our unpublished preliminary data, the profiles of coagulation biomarkers differ between AST and VM patients. This finding requires confirmation with a larger study population.
- Ongoing research on the specific effects of genetic mutations will further elucidate the pathophysiology of VMs and the co-regulation of angiogenesis and coagulation in VM patients.

## 7. Conclusions

Our research suggests that many intramuscular vascular lesions diagnosed as VMs but with unsatisfactory sclerotherapy outcome are not VMs by histology but are instead compatible with another distinct entity, designated as AST (I).

We found novel associations between coagulation biomarkers and VMs that are not explained by the prevailing theory of increased consumption, but reinforce the idea of a close relationship between coagulation and angiogenesis (II).

Superficial lesion location and use of ethanol are predisposing factors for sclerotherapy complications, most of which are local and conservatively manageable (III). Extensive morphology, critical locations, and blood coagulopathy are associated with severe, otherwise rare sclerotherapy complications (III, IV).

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## **10. Original publications**